CHARACTERIZATION AND ANTIMICROBIAL ANALYSIS OF CHITOSAN COMPOSITE BIODEGRADABLE FILMS WITH ADDITION OF GINGER ESSENTIAL OIL

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ABSTRACT

Innovative techniques of preserving food safety and structural integrity as well as complete biodegradability must be adopted. This research involves the development process in making the antimicrobial biodegradable film in order to restrain and inhibit the growth of spoilage and pathogenic bacteria that are contaminating food. The main objective of this research is to formulate the best film for food packaging, which has high thermal, antimicrobial and mechanical properties. The films were prepared by casting method. Chitosan were dissolved in 1% v/v acetic acid and yam starch by heat gelatinized, then PEG and ginger essential oil was added to the mix solution. After that, six analyses were tests for those films. The antimicrobial activity was determined by liquid culture test and agar plate test. The results showed that Sample C has highly antimicrobial properties in order to inhibit more *Bacillus subtilis* and *Escherichia coli* in liquid culture medium and has greatest clear zone on agar plate. The films morphology structure was observed using scanning electron microscopy (SEM). The results revealed that Sample C more smooth surface and compact structure. Chemical composition of the films was investigated using fourier transform infrared spectroscopy (FTIR) and revealed that starch, chitosan, essential oil and additives presence in the films. The thermal stability characterization using thermal gravimetric analysis (TGA) and differential scanning calorimetric (DSC) showed that Sample C has higher melting point and high heat resistance. In conclusion, Sample C is the best among three samples, prove that the addition of antimicrobial agent such as essential oil will give a better performance in film making.

ABSTRAK

Teknik inovatif untuk memelihara keselamatan makanan dan integriti struktur serta biodegradasi lengkap harus diambil. Penyelidikan ini melibatkan proses pembangunan dalam pembuatan filem biodegradasi antimikrob untuk menahan dan menghalang pertumbuhan pembusukan dan bakteria patogen yang mencemarkan makanan. Objektif utama penyelidikan ini adalah memformulasikan filem terbaik untuk bungkusan makanan, yang tinggi kestabilan terma, antimikrob dan sifat mekanikal. Filem-filem itu dibuat melalui kaedah *casting*. Chitosan dilarutkan di dalam 1% v / v asid asetat dan larutan tepung keladi dipanaskan, kemudian PEG dan minyak pati halia ditambah kepada larutan campuran. Kemudian, enam analisis diuji ke atas filem-filem tersebut. Aktiviti antimikrob ditentukan oleh *liqiud culture test* dan *agar plate test*. Keputusan menunjukkan Sampel C sangat antimikrob untuk menghalang pembiakan *Bacillus subtilis* dan *Escherichia coli* dalam medium kultur cecair dan mempunyai zon jelas terbesar di piring agar. Struktur morfologi filem diamati dengan *scanning electron microscopy* (SEM). Keputusan menunjukkan bahawa sampel C lebih halus permukaan dan berstruktur padat. Komposisi kimia filem diselidiki menggunakan *fourier transform infrared spectroscopy* (FTIR) dan mendedahkan keladi, chitosan, minyak pati halia dan aditif dalam filem. Karakterisasi kestabilan terma menggunakan *thermal gravimetric analysis* (TGA) dan *differential scanning calorimetric* (DSC), menunjukkan bahawa Sampel C mempunyai takat lebur yang lebih tinggi dan tahan haba. Kesimpulannya, Sampel C adalah yang terbaik di antara tiga sampel, membuktikan bahawa penambahan agen antimikrob seperti minyak pati halia akan memberikan prestasi yang lebih baik dalam pembuatan filem.

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LIST OF SYMBOLS

PEG	Polyethylene glycol
SEM	Scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
TGA	Thermo gravimetric analysis
DSC	Differential scanning calorimeter
% v/v	Percent volume per volume
λ	Wavelength
β	Heating rate
T	Temperature
T_M	Melting temperature

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Packaging is a modern technique for protecting food, increasing shelf life and safety. Furthermore, it facilities the sale and distribution of agricultural, industrial and consumer products, making distribution also possible over long distances. The package must prevent any contact between items and the surrounding, avoiding any undesired alterations and maintaining the purity and freshness of its contents. Moreover, it has the role of a communicative link between consumer and manufacturer, it must identify the contents, their quantity, the price, warning and other information that are carried on an applied label or directly imprinted on the packaging. Today, the basic materials of packages include, paper, paperboard, cellophane, steel, glass, wood, textiles and plastics. With 74% of the 13 billion m^2 market, plastic is the dominant flexible packaging material in Western Europe, according to a new Market power report. Total consumption of flexible packaging grew by 2.9% per year in 1992–1997, with the strongest growth in processed food and above average growth in chilled foods, fresh foods, detergent and pet foods. In this field plastics allow packaging to perform many necessary tasks providing many important properties such as strength and stiffness, barrier to oxygen transmission and moisture, resistance to food component attack and flexibility. Future growth will be 2% per year, as some markets reach maturity. The share of plastics in Western Europe will increase to 75.8% in 2002 (Avella *et al.*, 2001).

Nowadays, the largest parts of materials used in packaging industries are produced from fossil fuels and are practically nondegradable. For this, packaging materials for foodstuff, like any other short-term storage packaging material, represent a serious global environmental problem (Kirwan and Strawbridge, 2003). A big effort to extend the shelf life and enhance food quality while reducing packaging waste has encouraged the exploration of new bio-based packaging materials, such as edible and biodegradable films from renewable resources (Tharanathan, 2003). The use of these materials, due to their biodegradable nature, could at least to some extent solve the waste problem (Sorrentino *et al*., 2007). The escalating problems caused by non-degradable plastics have led to development of biodegradable plastics. The major advantages of biodegradable plastics are they can be composted with organic wastes and returned to enrich the soil; their use will not only reduce injuries to wild animals caused by dumping of conventional plastics but will also lessen the labor cost for the removal of plastic wastes in the environment because they are degraded naturally; their decomposition will help increase the longevity and stability of landfills by reducing the volume of garbage; and they could be recycled to useful monomers and oligomers by microbial and enzymatic treatment (Tokiwa and Calabia ,2007).

Antimicrobial packaging is gaining interest from researchers and industry due to its potential to provide quality and safety benefits. By means of the correct selection of materials and packaging technologies, it is possible to keep the product quality and freshness during the time required for its commercialization and consumption (Brown, 1992; Stewart, *et al*., 2002; Sorrentino *et al*., 2007). Currently, development is limited due to availability of antimicrobials and new polymer materials, regulatory concerns, and appropriate testing methods. With the advent of new materials and more information this may change. New coating/binder materials compatible with polymers and antimicrobials, functionalized surfaces for ionic and covalent links and new printing methods combined with encapsulation are examples of the technologies that will play a role in the development of antimicrobial packaging. Antimicrobials that can be attached or coated to films and rigid containers after forming to avoid high temperature and other processing issues will allow a wide range of compounds to be incorporated into polymers. These developments will require surfaces containing functional groups available for attachment. Physical methods to modify polymer surface (electron beam, ion beam, plasma and laser treatments) are emerging and pose potential for functionalizing inert surfaces such as those of PE, PET, PP and PS (Ozdemir, *et al*., 1999) HDPE and LLDPE have already been functionalized by graft polymerization with amide, amino and carboxyl groups in order to immobilize proteins and enzymes (Hayat *et al*., 1992; Sano *et al*., 1993; Wang and Hsiue, 1993). It has been suggested also that cross-linking edible films like calcium caseinate by gamma irradiation will find applications as supports for the immobilization of antimicrobials and other additives (Lacroix and Ouattara, 2000).

Future work will focus on the use of biologically active derived antimicrobial compounds bound to polymers. The need for new antimicrobials with wide spectrum of activity and low toxicity will increase. It is possible that research and development of ‘intelligent’ or ‘smart’ antimicrobial packages will follow. These will be materials that sense the presence of microorganism in the food, triggering antimicrobial mechanisms as a response, in a controlled manner. Antimicrobial packaging can play an important role in reducing the risk of pathogen contamination, as well as extending the shelf-life of foods; it should never substitute for good quality raw materials, properly processed foods and good manufacturing practices. It should be considered as a hurdle technology that in addition with other non-thermal processes such as pulsed light, high pressure and irradiation could reduce the risk of pathogen contamination and extend the shelf-life of perishable food products. Participation and collaboration of research institutions, industry and government regulatory agencies will be key on the success of antimicrobial packaging technologies for food applications (Appendini and Hotchkiss, 2002).

1.2 Problem Statement

Nowadays, about 150 million tons of plastic are produced annually all over the world, and the production and consumption continue to increase (Parra *et al.*, 2004). Most of these plastics are crude oil based. In addition, handling of plastic waste associated with serious environmental pollution problem due to waste disposal and nondegraded polymers. Therefore, the use of agricultural biopolymers that are easily biodegradable not only would solve these problems, but would also provide a potential new use for surplus farm production (Okada, 2002; Pavlath and Robertson, 1999; Scott, 2002 and Salleh *et al*. 2007). The environmental impact caused by the excessive quantity of non-degradable waste materials discarded every day is a matter of great concern. This reality is stimulating a great R&D effort to develop new biodegradable packing materials that can be manufactured with the utilization of environmentally friendly raw materials (Avérous *et al.*, 2001 and Galdeano *et al*. 2009).

The use of protective coatings and suitable packaging by the food industry has become a topic of great interest because of their potentiality for increasing the shelf life of many food products (Ahvenainen, 2003; Coles *et al*., 2003; Giles and Bain, 2001; Hernandez *et al*., 2000). By means of the correct selection of materials and packaging technologies, it is possible to keep the product quality and freshness during the time required for its commercialization and consumption (Brown, 1992; Stewart *et al*., 2002). A big effort to extend the shelf life and enhance food quality while reducing packaging waste has encouraged the exploration of new bio-based packaging materials, such as edible and biodegradable films from renewable resources (Tharanathan, 2003). The use of these materials, due to their biodegradable nature, could at least to some extent solve the waste problem. However, like conventional packaging, bio-based packaging must serve a number of important functions, including containment and protection of food, maintaining its sensory quality and safety, and communicating information to consumers (Robertson, 1993 and Sorrentino *et al*., 2007).

The performance expected from bioplastic materials used in food packaging application is containing the food and protecting it from the environment and maintaining food quality (Arvanitoyannis, 1999). It is obvious that to perform these functions is important to control and modify their mechanical and barrier properties that consequently depend on the structure of the polymeric packaging material. In addition, it is important to study the change that can occur on the characteristics of the bioplastics during the time of interaction with the food (Scott, 2000 and Siracusa *et al*., 2008).

So, the development of this research is due to the handling problem of non-degradable plastics packaging waste in the world and the high production cost of the biodegradable plastics packaging that consumes to global warming and non-friendly environment issues. In order to preserve food safety and to sustain the environment, the advances in film making have been study. This research involves the development process in making the antimicrobial biodegradable film in order to restrain and inhibit the growth of spoilage and pathogenic microorganisms that are contaminating food.

The significant of the research is to use an alternative method to petroleum-based plastic for plastic packaging material. In consequent, it will decrease the soil pollution and environment problem by producing the biodegradable plastics. By applying antimicrobial agent, it will give the effect to the extension of the shelf-life of the food and the maintenance or even improvement of its quality. These advances in the technology of film production will be future interest and the global market demand with a special emphasis on safety concerns and assessment.

1.3 Research Objective

The objective of this research are to formulate the best film for food packaging, which is high thermal stability, highly antimicrobial, highly mechanical properties and smooth film's surface from the mixture of chitosan and yam starch with addition of polyethylene glycol (PEG) and ginger essential oil. Another objective is characterization of film fabricated by various methods which are:

a. Scanning Electron Microscopy (SEM)
b. Fourier Transform Infrared (FTIR)
c. Thermo Gravimetric Analysis (TGA)
d. Differential Scanning Calorimeter (DSC)
e. Antimicrobial Analysis

1.4 Scope of Study

In order to achieve the objective, scopes have been identified in this research. The scopes of this research are list as below:

a. Film preparation from the chitosan-yam starch solution by casting method.
b. Evaluation the antimicrobial effectiveness toward *Bacillus subtilis* and *Escherichia coli* on the film packaging which representing gram positive and gram negative bacteria.
c. Characterize the film by using various methods which are Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR), Thermo Gravimetric Analysis (TGA), and Differential Scanning Calorimeter (DSC).

CHAPTER 2

LITERATURE REVIEW

2.1 Biodegradable Film

The materials most used for food packaging are the petrochemical-based polymers, due to their availability in large quantities at low cost and favourable functionality characteristics, such as, good tensile and tear strength, good barrier properties to O_2 and heat sealability (Tharanathan, 2003). However, these materials are totally non-biodegradable, leading to serious ecological problems. As a consequence, the consumer demand has shifted to eco-friendly biodegradable materials, especially from renewable agriculture by-products, food processing industry wastes and low cost natural resources. The biopolymers commonly used to produce films are carbohydrates, often vegetal starchy and pectic materials and proteins, vegetal and animal (Vermeiren *et al*., 1999 and Alvarez, 2000). Usually, these biopolymers require that their mechanical and rheological properties be improved by molecular restructuring or by the inclusion of food grade additives. In addition to the appropriate mechanical properties, the films must have also the adequate permeability to water vapour and gases. The specific barrier requirements of the packaging depend upon the products characteristics and the intended end-use application. In the case of a packaged product whose deterioration is related to its moisture content, the barrier properties of the package relating to water vapour will be of major importance in extending shelf life. Similarly, the oxygen concentration in a permeable package will affect the rate of oxidation of nutrients such as vitamins, proteins and fatty acids. The required specific permeability properties of the films can be obtained by inclusion of inert impermeable barriers and/or reactive compounds in the polymer matrix. The inert barriers can reduce permeability by increasing the diffusion path, while the reactive compounds interact selectively with the diffusing species increasing the time before a significant permeability occurs (Alves *et al*., 2006).

2.2 Biocomposite

Ecological concerns have resulted in a renewed interest in natural and compostable materials, and therefore issues such as biodegradability and environmental safety are becoming important. Tailoring new products within a perspective of sustainable development or eco-design is a philosophy that is applied to more and more materials. It is the reason why material components such as natural fibres, biodegradable polymers can be considered as interesting and environmentally safe and can be used as alternatives for the development of new biodegradable composites. When it comes to improvements in edible film technologies, most research has addressed film formulations using various combinations of edible materials. Two or more materials can be combined to improve gas exchange, adherence to coated products, or moisture vapor permeability properties (Baldwin et al., 1995). Biodegradable composites consist of biodegradable polymers as the matrix material and biodegradable fillers, usually biofibres. Since both components are biodegradable, the composite as the integral part is also expected to be biodegradable (Mohanty *et al*., 2000). (Averous and Boquillon, 2004).

Composite films consisting of lipids and a mixture of proteins or polysaccharides take advantage of the individual component properties. In doing so, these individual or combined films can be applied as emulsions or bilayer films (Cutter and Sumner, 2002). Additionally, plasticizers can be used to modify film mechanical properties, thereby imparting desirable flexibility, permeability, or solubility to the resulting film (Ben and Kurth, 1995). For example, adding glycerol, polyethylene glycol, or sorbitol to a film composition can reduce brittleness (Ben and Kurth, 1995). In another example of composite films, a combination of vegetable oils, glycerin, citric acid, and antioxidants prevented rancidity by acting as a moisture barrier, restricting oxygen transport, and serving as a carrier for antioxidants to various foods (Baldwin *et al*., 1995; Cutter and Sumner, 2002). In another study, barrier properties were determined for caseinate films that were treated with a lipid or an enzyme and held at 4°C and 90% relative humidity (Ben and Kurth, 1995). Lipid addition notably improved moisture barrier properties, but the films appeared slightly cloudy, such that when these particular films were applied to meat surfaces, the appearance of the meat surface was unacceptable (Ben and Kurth, 1995, Cutter, 2006).

2.3 Biopolymer or Bio-based Polymer

The biopolymers commonly used to produce films are carbohydrates, often vegetal starchy and pectic materials and proteins, vegetal and animal (Vermeiren *et al*., 1999 and Alvarez *et al*., 2000). Usually, these biopolymers require that their mechanical and rheological properties be improved by molecular restructuring or by the inclusion of food grade additives. In addition to the appropriate mechanical properties, the films must have also the adequate permeability to water vapour and gases. The specific barrier requirements of the packaging depend upon the products characteristics and the intended end-use application. In the case of a packaged product whose deterioration is related to its moisture content, the barrier properties of the package relating to water vapour will be of major importance in extending shelf life. Similarly, the oxygen concentration in a permeable package will affect the rate of oxidation of nutrients such as vitamins, proteins and fatty acids (Alves *et al*., 2006).

Typically, bio-based polymers or biopolymers are developed from renewable resources (Comstock *et al*., 2004; Weber *et al*., 2002). Examples of renewable resources used in the manufacture of these types of polymers include polysaccharides such as starch, alginates, pectin, carrageenans, and chitosan/chitin, proteins such as casein, whey, collagen, gelatin, corn, soy, and wheat, and lipids such as fats, waxes, or oils (Comstock et al., 2004; Cutter and Sumner, 2002). Polymers, such as polylactate (PLA) or polyesters also may be synthesized from biologically-derived monomers, while microorganisms also can produce polymers such as cellulose, xanthan, curlan, or pullulan (Comstock *et al*., 2004; Kandemir *et al.*, 2005). Researchers also have further categorized biopolymers based on the ability to be compostable or biodegradable (Comstock *et al.*, 2004). It is important to note that while some bio-based packaging materials may be biodegradable, not all biodegradable materials are bio-based (Weber *et al*., 2002 and Cutter, 2006).

Recent technological advances also have allowed biopolymers to be processed similarly to petroleum-based plastics, whether in sheets, by extrusion, spinning, injection molding, or thermoforming (Comstock *et al*., 2004). Notable advances in biopolymer production, consumer demand for more environmentally-friendly packaging, and technologies that allow packaging to do more than just encompass the food are driving new and novel research and developments in the area of packaging for muscle foods (Cutter, 2006).

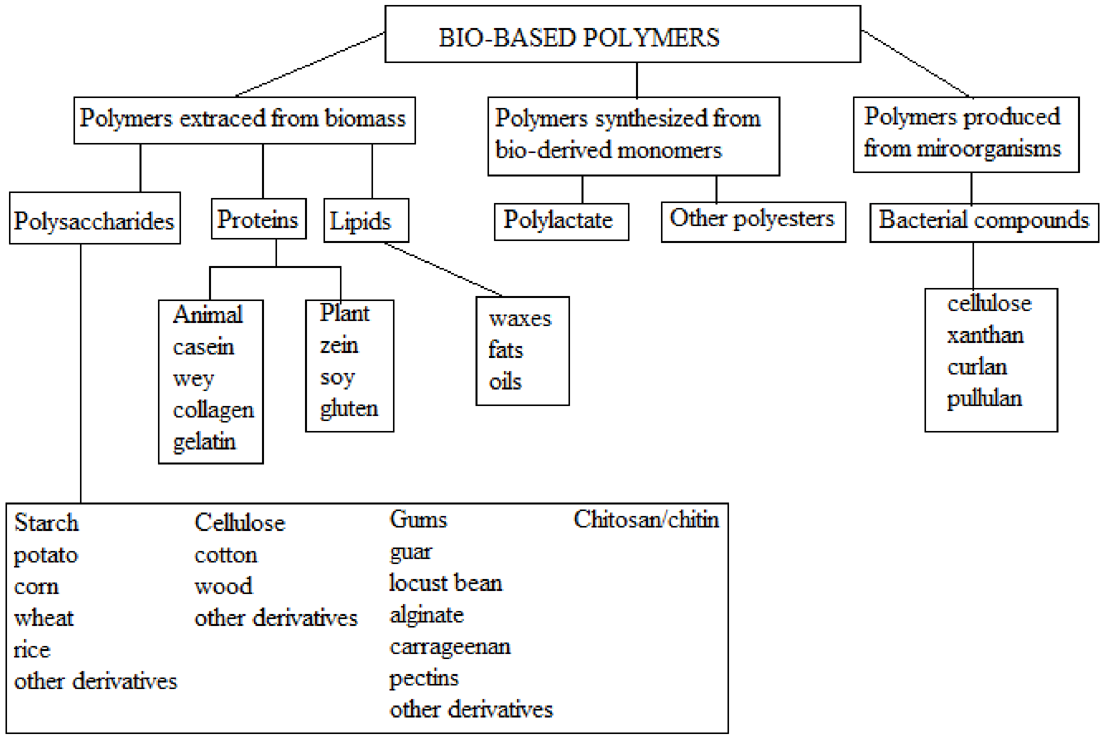

Figure 2.1 Different categories of bio-based materials (adapted from Weber *et al*., 2002).

2.4 Antimicrobial Packaging

The demand for minimally processed, easily prepared and ready-to-eat 'fresh' food products, globalization of food trade, and distribution from centralized processing pose major challenges for food safety and quality. Recent food-borne microbial outbreaks are driving a search for innovative ways to inhibit microbial growth in the foods while maintaining quality, freshness, and safety. One option is to use packaging to provide an increased margin of safety and quality. The next generation of food packaging may include materials with antimicrobial properties. These packaging technologies could play a role in extending shelf-life of foods and reduce the risk from pathogens. Antimicrobial polymers may find use in other food contact applications as well.

The use of bio-based, polymer-based films as antimicrobial delivery systems to reduce undesirable bacteria in foodstuffs is not a novel concept. Various approaches have been proposed and demonstrated for the use of these films to deliver compounds to a variety of food surfaces, including muscle foods. As mentioned previously, these types of films, gels or coatings are receiving considerable attention since they satisfy consumers' demands for products made from sustainable materials and/or recyclability (Durango et al., 2006 and Cutter, 2006).

Antimicrobial packaging is a form of active packaging. Active packaging interacts with the product or the headspace between the package and the food system, to obtain a desired outcome (Labuza and Breene, 1989; Rooney, 1995; Brody, Strupinsky and Kline, 2001). Likewise, antimicrobial food packaging acts to reduce inhibit or retard the growth of microorganisms that may be present in the packed food or packaging material itself (Appendinia *et. al*, 2002). Direct addition of antimicrobial substances into food formulations or onto food surfaces may not be sufficient to prevent the growth of pathogenic and spoilage microorganisms as antimicrobial substances applied could be partially inactivated or absorbed by the food systems (Ouattara *et al.,* 2000). Antimicrobial films render sustained release of antimicrobial substances onto the food surface and compensate for the partial inactivation or absorption of them by food systems (Siragusa and Dickson, 1992).

2.4.1 Type of Antimicrobial Packaging

From the Journal of Review of Antimicrobial Food Packaging (2002), the writers had determined the form of Antimicrobial packaging. Below are several forms of Antimicrobial packaging which are:

a. Addition of sachets/pads containing volatile antimicrobial agents into packages.

b. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers.
c. Coating or adsorbing antimicrobial agents into polymer surfaces.
d. Immobilization of antimicrobial agents to polymers by ion or covalent linkages.
e. Use of polymers that are inherently antimicrobial.

In this research, I will focus on incorporation of volatile and non-volatile antimicrobial agents directly into polymers. The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods were a large portion of spoilage and contamination occurs. For example, intact meat from healthy animals is essentially sterile and spoilage occurs primarily at the surface. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. Table below shows the different antimicrobial agents directly incorporate with different polymers for antimicrobial food packaging.

Table 2.1: Antimicrobial incorporated directly into polymers used for food packaging (Appendini and Hotchkiss, 2002).

Antimicrobials incorporated directly into polymers used for food packaging			
Antimicrobials	Polymer/ carrier	Main target microorganisms	References
Organic acids / anhydrides: Propionic, benzoic, sorbic, acetic, lactic, malic	Edible films, EVA, LLDPE	Molds	Guilbert(1988), Baron & Summer(1993), Torres & Karel(1985), Devlieghre, Vermeiren, Hockstal & Debevere (2000), Weng & Hotchkiss(1993)
Inorganic gases: Sulfure dioxide, chlorine	Various polyolefins	Molds, Bacteria, Yeasts	CSIRO (1994) Wellinghoff (1995)

dioxide			
Metals: Silver	Various polyolefins	Bacteria	Ishitani (1995)
Fungicide: Benomyl, imazalil	LDPE	Molds	Weng(1992) Padgett, Han & Dawson (1998)
Bacteriocius: Nisin, pediocins, lacticin	Edible films, cellulose, LDPE	Gram-positive bacteria	Siragusa, Cutter & Willet (1999) Scanell, Hill, Ross, Mars, Hartmeier & Areadt (2000)
Enzymes: Lysozyme, glucose oxidase	Cellulose acetate, PS	Gram-positive bacteria	Appendini and Hotchkiss (1997) Padget et. Al (1998)
Chelating agents: EDTA	Edible films	Gram-negative bacteria	Padget et. Al (1998)
Spices: Cinamic, caffeic, p-coumaic acids Horseradish (allylisothiocynate)	Nylon/PE, cellulose	Molds, Bacteria, Yeast	Hoshino, Ijima, Hayashi & Shibata (1998) Anon (1995), Nielsen & Rios (2000)
Essential oils(plant extracts): Grapefruit seed extract, bamboo powder, Rheum palmatum, Coptis chinesis extracts	LDPE, cellulose	Molds, Bacteria, Yeast	Lee, Hwang & Cho (1998) Imakura, Yamada & Fukuzawa (1992) Oki(1998), Chung, Cho & lee (1998) Hong et al. (2000)
Parabens: Propylparaben, ethylparaben	Clay-coated cellulose LDPE	Molds	Katz (1998) Dobies et al. (1998)
Miscellaneous: Hexamethyl-eneteteamin	LDPE	Yeasts, anaerobes and acrobes	Devlieghere et al. (2000)
Abbreviations: EVA(ethylene vinyl acetate); LLDPE (linear low density polyethylene); LDPE (low density polyethylene); PS (polystyrene); PE (polyethylene)			

2.5 Chitosan

Chitosan is a carbohydrate polymer that can be derived from crustacean seafood wastes such as shells of crabs, shrimps and crawfish. Chitosan has a wide range of applications in diverse fields ranging from medical sutures and seed coatings to dietary supplements and coagulants for waste treatment. Physicochemical properties of chitosans and their functionalities are affected by their sources (Rhazi *et al*., 2004). Chitosan is the N-deacetylated derivative of chitin; although this N-deacetylation is almost never complete, this could be defined as chitin sufficiently deacetylated to form soluble amine salts. The required degree of deacetylation to obtain a soluble product must be 80–85% or higher. Chitosan products are highly viscous, resembling natural gums (Peniston and Johnson, 1980). The physico-chemical and biological properties of chitosan justify its introduction in food formulations once it could improve nutritional, hygienic and/or sensory properties, because of its emulsifying, antimicrobial, antioxidant and gelling properties, while also acting as a functional fiber. Chitosan's safety can be evaluated by its remarkably high lethal doses (1.6 g/kg of body weight in rats), being comparable to those of sugar and even less toxic than salt. For all these reasons, chitosan has been accepted as a dietary supplement or a food additive in many countries (e.g. Italy, France, Norway, Poland, United States of America, Argentine, Japan and Korea) (Argullo´ *et al*., 2004 and Park *et al*., 2002). (Nadarajah, 2005).

2.5.1 Sources of Chitosan

Chitosan is converted from chitin, which is a structural polysaccharide found in the skeleton of marine invertebrates, insects and some algae. Chitin is perhaps the second most important polysaccharide after cellulose and is an abundantly available renewable natural resource. The aquatic species that are rich in chitinous material (10-55 % on a dry weight basis) include squids, crabs, shrimps, cuttlefish and oysters. Mucoraceous fungi, which are known to contain chitin and the deacetylated derivate, chitosan, in cell walls (22 to 44%), have been used for commercial chitin production